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Controlled Temperature Tissue Fusion:
Ho:YAG Laser Welding of Rat Intestine In Vivo, Part Two

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Background and Objective: **Temperature feedback control (TFC) during laser-assisted tissue welding was implemented to eliminate exponential increases in the rate of denaturation associated with rapidly increasing temperatures. This study was undertaken to investigate and compare the weld strengths and healing responses of laser welded enterotomies with and without TFC using a cw Ho:YAG laser and to examine the effects of wavelength on weld strength and histology. The Ho:YAG experimental results were compared with a similar study using cw argon ion laser irradiation.**
Study Design/Materials and Methods: **An automated system was developed for temperature feedback controlled laser irradiation. An experimental device incorporating co-aligned laser delivery and temperature detection was used to perform cw Ho:YAG laser-welded enterotomies (with and without TFC). The weld strength and histology of laser welded and control sutured enterotomies were compared in an in vivo rat model (Ho:YAG, n = 42; argon, n = 41). Animals were sacrificed at 1, 3, 7, and 21 days postoperatively and the anastomotic site was removed for bursting/leaking pressure measurements and histological examination.**
Results: **Argon and Ho:YAG laser-welds with and without TFC and the control sutured anastomoses healed comparably, although wound abscesses were more prevalent in the Ho:YAG group leading to delay in mucosal healing. Laser-welded anastomoses without TFC were associated with more spontaneous ruptures and leaks (argon: 4/6 ruptures; Ho:YAG: 1/4 leak, 2/4 ruptures, & 1/3 stenosis) during the survival period than those with TFC (argon: 1/3 leak; Ho:YAG: 1/5 rupture). Bursting pressures of the Ho:YAG welds were weaker at 1 week than the ar-**

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gon welds, but by 3 weeks, laser welds and suture anastomoses were equally strong.
Conclusion: **From the spontaneous failure rates encountered, it is believed that TFC improves the quality and stability of laser-assisted enterotomy closures in surviving animals. However, TFC does not provide a satisfactory method to identify completion of a weld. Lasers Surg. Med. 21:278–286, 1997.**

Key words: anastomosis; dosimetry; feedback control; Ho:YAG laser; infrared sensor; intestine; quasi-constant temperature control; rate process; thermal damage control; temperature feedback; tissue fusion; tissue welding

INTRODUCTION

Over the last few years, we tested the hypothesis that tissue surface temperature is an indicator of tissue status during laser-assisted tissue welding (LTW). Temperature feedback control of tissue welding was used in in vitro and in vivo models. We found that laser-assisted anastomoses created in isolated segments of fresh canine jejunum in vitro were optimally strong at 90–95°C feedback control temperatures [1]. Recently, we further tested our hypothesis by welding rat small intestine segments in vivo with argon laser radiation under temperature feedback control (TFC) at 90°C control temperature and without temperature feedback [2]. Although the healing responses did not differ, TFC was associated with less spontaneous ruptures of the anastomotic sites during the first 24-hour postoperative period. The present study was undertaken to compare the weld strength and healing response of laser-welded enterotomies with and without TFC using a continuous wave (cw) Ho:YAG laser. The results are compared with those from our previous argon ion laser study to examine the effects of wavelength on weld strength and histology.

The primary chromophore in intestine segments for absorption of the Ho:YAG wavelength of 2.09 μm is water, whereas the primary chromophore for the argon wavelengths is blood. In the absence of blood, the penetration depth of argon radiation is about the same as that of the Ho:YAG laser. The penetration depth (1/reduced attenuation coefficient) in soft tissue for both 500 nm light and 2.09 μm light is 300–400 μm [3,4]. However, the penetration depth of the argon light is governed by absorption and scattering, whereas the penetration depth of the Ho:YAG light is determined solely by absorption. That is, the absorption coefficient of tissue at 500 nm is much smaller than that at 2.09 μm. Correspondingly, for the same irradiance, the rate of heat generation at the surface for 2.09 μm is larger than the rate of heat generation at 500 nm. For similar rates of heat generation at the two wavelengths, the irradiance at 500 nm must be much larger than the irradiance at 2.09 μm.

MATERIALS AND METHODS

Surgical Procedures

Forty-six female Wistar rats (Harlan Sprague Dawley, Houston, TX) ages 6–7 weeks, were used for experimentation. The surgical procedures were the same as in our previous study [2] except that (1) the animals were given honey water until 2–3 hours preoperatively to prevent fasting animals from eating the bedding in their cages, and (2) full anesthesia was introduced with a mixture of ketamine, rompun, and atropine (9:5:1), 0.15 ml/100 gr. intramuscularly.

System Components

The experimental setup of the controlled temperature laser delivery system and system components have been described in detail in a previous work concerning argon laser welding of rat intestine in vivo [2].

In this study, a cryogenically cooled cw Ho:YAG laser beam that operates at 2.09 μm (Rare Earth Medical Lasers, West Yarmouth, MA) is used. Surface temperature signals are collected and recorded from the FOV of the co-aligned device at the center of the 2 mm diameter laser spot on the focal plane of the device.

Experimental Procedure

Intestinal welding was performed with and without TFC at 90°C. Using a low power HeNe alignment beam, the enterotomy was brought into the focal plane of the co-aligned device. Laser power and laser delivery efficiency of the device were 500 mW and 60%, respectively, resulting in an irradiance of 16 W/cm^2 at the focal plane of the

co-aligned device. The irradiance for our previous experiment using an argon laser was 28 W/cm² [2].

Once the rats were fully awake from anesthesia, they were placed individually in small cages and given honey water. Their regular diet was gradually resumed ~24 hours postoperatively depending on their physical condition.

Histological Examination

Following standard histological procedures described earlier [2], the location and extent of thermal damage, including collagen hyalinization, collagen and smooth muscle birefringence loss, and coagulative and hemorrhagic tissue necrosis, were evaluated in the 1-day specimens. Wound healing configuration, composition, and progression were observed at 3 days, 1 week, and 3 weeks. Quantitative histopathologic examination included measurements of (1) the extent of coagulative and hemorrhagic necrosis from the wound edges to the boundary between the necrotic and viable tissue, and (2) gap widths that formed between the wound edges. Qualitative histologic features and quantitative measurements of the argon ion [2] and Ho:YAG experimental groups were compared.

Data Analysis

Statistical analysis of BLP measurements was carried out using Microsoft Excel® 4.0. Data were plotted on KaleidaGraph™ 3.0. Data were presented as averages with standard deviations and ranges. Bursting pressures of Ho:YAG welds were compared to comparable specimens welded with an all lines argon ion laser and controls [2].

EXPERIMENTAL RESULTS

Laser-assisted intestinal anastomoses provided an immediate fluid-tight seal when compared to control sutured anastomoses. The average "total exposure time" required to perform laser-assisted anastomoses with and without TFC differed significantly. The "total exposure times" for Ho:YAG welding was significantly shorter than the corresponding exposure times for our previous argon anastomoses (see Table 1). In this study, "total exposure time" included laser on-and-off times during TFC and non-TFC irradiation. An example of temperature histories during laser-assisted anastomosis formed with and without TFC is shown in Figure 1. Temperature profiles without TFC were highly erratic owing to excessive heating during solely visual control.

TABLE 1. Total Times: Comparisons of Argon and Ho:YAG Laser-Assisted Intestinal Anastomoses

	Assisted anastomosis with TFC [sec]	Assisted anastomosis without TFC at 90°C [sec]
Ho:YAG	106 ± 40	63 ± 25
Argon Ion	142 ± 36	86 ± 20

Some Ho:YAG laser-assisted anastomoses that were intact at the completion of welding did not survive (see Table 2). Most of the complications were evident in the first 36 hours after operation. Two animals died within 24 hours due to spontaneous rupture of the anastomosis leading to fecal peritonitis. In surviving rats examined at 1 day, one anastomosis had a small leak of fecal material and another anastomosis that was intact upon removal broke apart when being mounted on the bursting pressure apparatus. One other animal was remarkably debilitated at the time of euthanasia (3 weeks). At autopsy, a severe stenosis that reduced the lumen to ~2 mm was present at the anastomotic site and the proximal bowel was dilated.

Out of a total of 14 animals operated with TFC, one animal's anastomosis was accidentally overexposed away from the wound edge and a large area of tissue shrinkage was observed. This weld ruptured spontaneously 24 hours postoperatively. Another intact 1-day anastomosis broke with handling at harvest. None of the 12 animals whose enterotomies were closed with sutures suffered complications.

Comparisons of the incidence and types of complications of the argon [2] and Ho:YAG laser-assisted rat intestine anastomoses showed that spontaneous rupture and leakage were common to anastomoses produced with both lasers (see Table 2). Nearly all complications occurred within the first 72 hours. The total number of complications including rupture and leakage was greater in the anastomoses formed without TFC (9/29 or 31%) compared to those formed with TFC (5/28 or 18%). Comparisons of complications based on wavelength (argon, λ = 488–514 nm and Ho:YAG, λ = 2.09 μm) show no significant differences between argon and Ho:YAG anastomoses with or without TFC.

Bursting Pressure Tests

The bursting/leaking pressure measurements of Ho:YAG laser-assisted intestinal anastomoses and suture controls are shown in Figure 2.

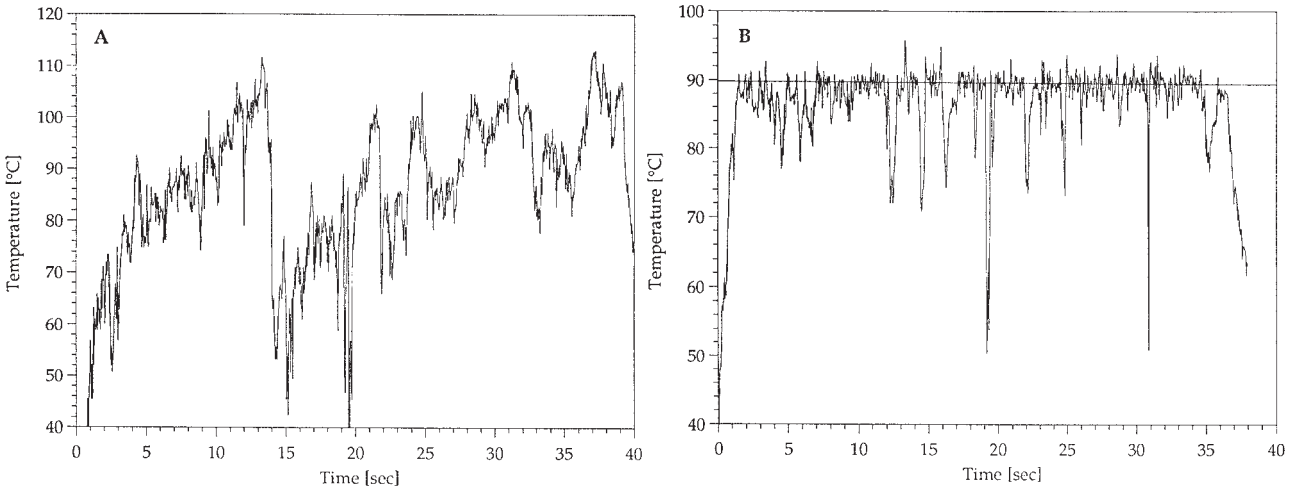

Fig. 1. A comparison of temperature histories of cryogenically cooled Ho:YAG laser-assisted rat intestinal anastomoses, **(A)** laser-assisted without thermal feedback control, **(B)** laser-assisted without thermal feedback control. The variations (particularly, low temperatures) about 90°C control temperature in (a) were due to continuous moving of the enterotomy during "brush welding." A large decrease in temperature occurs when an untreated portion is moved under the detector field of view.

TABLE 2. Complications: Comparisons of Argon and Ho:YAG Laser-Assisted Rat Intestinal Anastomoses

	Day 1	Day 3	1 Week	3 Weeks
Suture:				
both groups	6/6 intact	6/6 intact	6/6 intact	6/6 intact
Argon laser				
TFC	1/3 leaked[a]	2/5 broke[b]	3/3 intact	3/3 intact
No TFC	3/3 intact	4/6 ruptured	3/3 intact	3/3 intact
Ho:YAG laser				
TFC	1/5 ruptured 1/5 broke[b]	3/3 intact	3/3 intact	3/3 intact
No TFC	2/6 ruptured 1/6 leaked[a] 1/6 broke[b]	3/3 intact	2/2 intact	1/3 stenotic

[a]Leak of fecal material when examined *in situ* before removal from animal.
[b]Broke with handling during harvest or mounting on bursting pressure apparatus.

At 1 day, all anastomoses (sutured, laser-assisted with and without TFC) were very weak. Their average BLP's were 52 ± 10, 40 ± 16, and 43 ± 21 mm Hg, respectively. However, as reflected by the relatively large standard deviations and ranges, there were no statistically significant differences among the three groups.

At 3 days, laser-welded anastomoses with TFC (85 ± 22 mm Hg) were about as strong as sutured controls (90 ± 10 mm Hg). Laser-welded anastomoses without TFC (58 ± 16 mm Hg) remained significantly weaker than suture controls ($P < 0.05$). BLPs in specimens without TFC ranged from 40 to 70 mm Hg, whereas the range with TFC was 60–100 mm Hg.

At 1 week, sutured anastomoses (247 ± 23 mm Hg) were as strong as intact bowel (255 ± 36 mm Hg), yet laser-welded anastomoses with (150 ± 17 mm Hg) and without (160 ± 57 mm Hg) TFC were still relatively weak. Although the BLPs of the laser-assisted anastomoses with TFC were weaker than the suture controls, the BLPs of the TFC bonds approached those of the minimum bursting pressure in intact rat intestine (see Fig. 2). Up to this time, all laser-assisted anastomoses burst open or leaked at or very close to the stay sutures. Beyond day 7, the bonds burst open at locations other than the stay sutures.

At 3 weeks, all anastomoses were mechanically as strong as intact bowel.

Comparisons of the bursting pressures of the argon ion and Ho:YAG laser-assisted anastomoses showed that the mechanical strengths of the 1-week Ho:YAG bonds (with TFC, 150 ± 17 mm

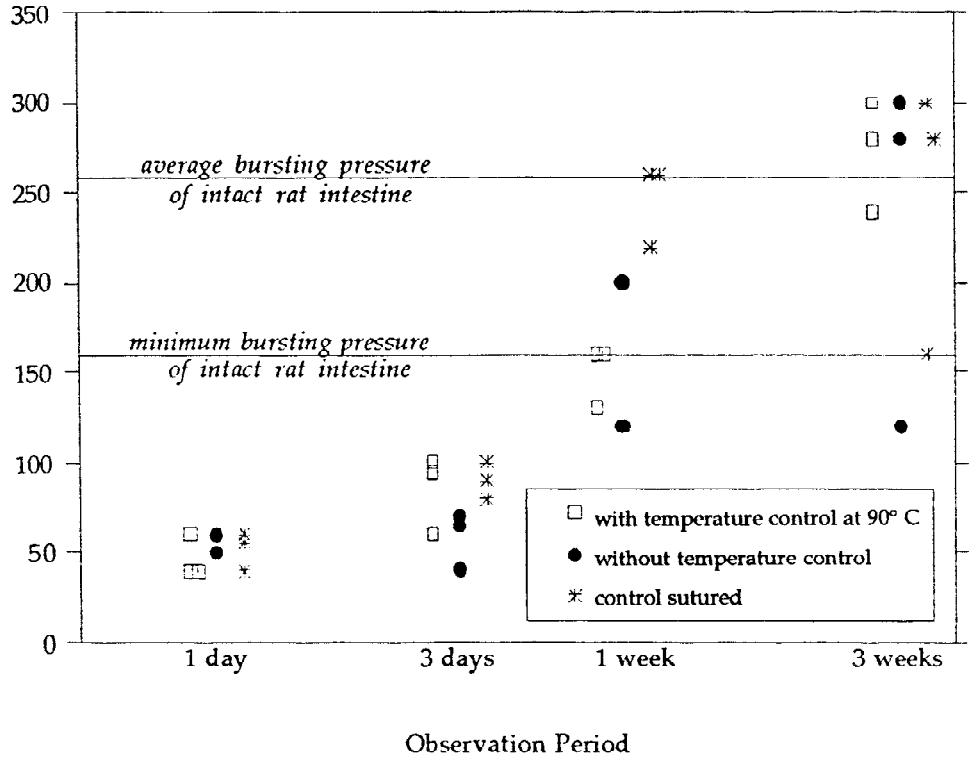

Fig. 2. Bursting/leaking pressures of Ho:YAG laser-assisted rat small intestinal anastomoses suture controls.

Hg; without TFC, 160 ± 57 mm Hg) were significantly less than those of the 1-week argon ion bonds (with TFC, 253 ± 21 and without TFC, 213 ± 31) (Table 3). Otherwise, argon and Ho:YAG bond strengths were similar with marked variation among the various laser-assisted groups compared to the suture controls.

HISTOLOGY RESULTS

At 1 day, the anastomotic sites are comparable for all three anastomotic techniques except for the foci of mural coagulative necrosis at the anastomotic ends of the laser-assisted welds with and without TFC (see Fig. 3). These pale, acellular coagulated end zones are separated from the adjacent tissue by a sharp boundary. Some 1-day specimens are further distorted by focal mural hemorrhagic necrosis adjacent to the anastomoses, suggesting that the blood supply to the anastomotic area has been compromised. The ends of the anastomoses have parted to form small gaps close to the sutures and large gaps away from the sutures in all specimens. Frequently, the gaps are filled by the fat tissues of the adjacent mesentery or omentum that is bound to the serosal and necrotic ends of the welds by fibrinous exudates. The luminal wound gap surfaces are covered with fecal material intermixed with purulent exudates of necrotic tissue, bacterial clumps, fibrin, and inflammatory cells. Sometimes deeper abscesses have formed in the omentum or mesentery. Very early healing inflammatory and mucosal regenerative responses are present at the wound edges.

At 3 days, the foci of coagulative necrosis and hemorrhagic infarcted bowel wall have disappeared, suggesting that slough of the dead tissue contributes to the widening of the anastomotic gaps [Fig. 4]. In some specimens, the omental and mesenteric abscesses have enlarged. No differences are seen among the different anastomotic methods. Early granulation tissue formation and glandular epithelial regeneration mark the early reparative changes of wound healing.

At 1 week, vascular and early fibrous wound tissue has begun to replace the fat in the mesentery and adherent omentum and the fibrinous inflammatory exudates that once bound the omentum to the bowel. The luminal surfaces of the omentum filling the gap are still composed of necrotic tissue intermixed with inflammatory exudates and fecal material. The glandular epithelium continues to grow and a few irregular glands are being regenerated.

TABLE 3. Bursting Pressures: Comparisons of Argon and Ho:YAG Laser-Assisted Intestinal Anastomoses

	Day 1 [mm Hg]	Day 3 [mm Hg]	1 Week [mm Hg]	3 Weeks [mm Hg]
Suture control				
Argon group	38 ± 19	60 ± 35	263 ± 32	293 ± 12
Ho:YAG group	52 ± 10	90 ± 10	247 ± 23	247 ± 76
Argon laser				
TFC	39 ± 18	47 ± 6	253 ± 21	277 ± 21
No TFC	43 ± 21	70 ± 14	213 ± 31	260 ± 20
Ho:YAG laser				
TFC	40 ± 16	85 ± 22	150 ± 17	273 ± 31
No TFC	43 ± 21	58 ± 16	160 ± 57	233 ± 99

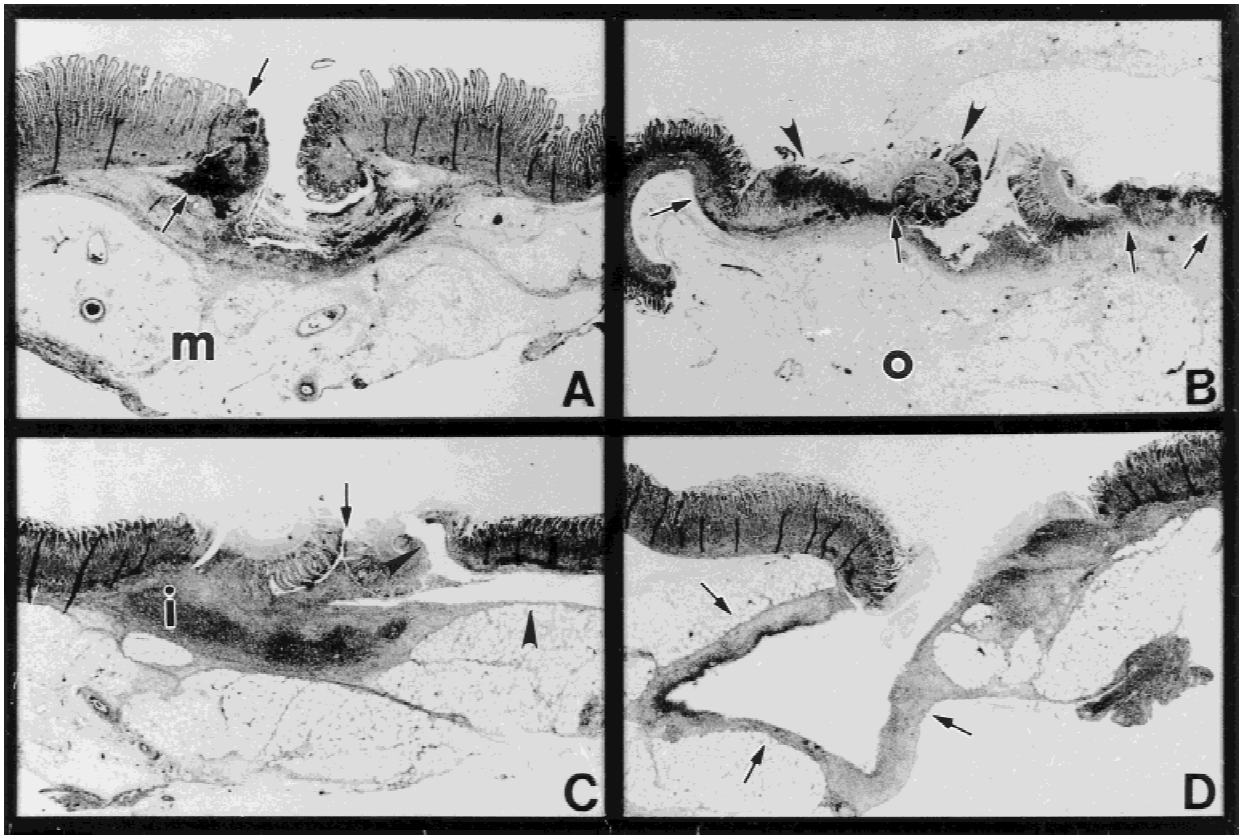

Fig. 3. 1 Day Ho:YAG laser assisted rat intestine anastomoses. **A.** Suture control anastomoses. Focal hemorrhagic necrosis (arrow) distorts one wound edge. A gap between the wound edges exposes the underlying hemorrhagic and inflamed fat tissue of the exposed mesentery (m). **B.** Ho:YAG without TFC. The boundaries between the pale thermally coagulated wound edges (arrow heads) and the zone of transmural hemorrhagic necrosis (arrows) are sharp. The omentum (o) adheres to the bowel serosal covering the gap between the wound edges. **C.** Ho:YAG with TFC. This specimen was collected close to one of the stay sutures; therefore the wound gap (arrow) is small. The omentum is attached to the serosal surface with hemorrhagic inflammatory exudates (i) of fibrin and inflammatory cells. The prominent cleft (arrow heads) represents the plane of rupture extending through the tract made by the suture (lost in tissue processing) to the mechanically weak inflammatory omental-serosal bond. **D.** Ho:YAG with TFC. A large abscess (arrows) extends from the wound gap deep into the adherent omentum. (Hematoxylin and eosin stain. Original magnification: 6.25×).

At 3 weeks, the scar tissue filling the anastomotic gaps contains more collagen and fewer inflammatory cells than before. The wound gaps have become smaller, but luminal surfaces still have not been completely covered with regenerated glandular mucosa [Fig. 4].

Comparisons of the argon [2] and Ho:YAG laser-assisted bonds with and without TFC and

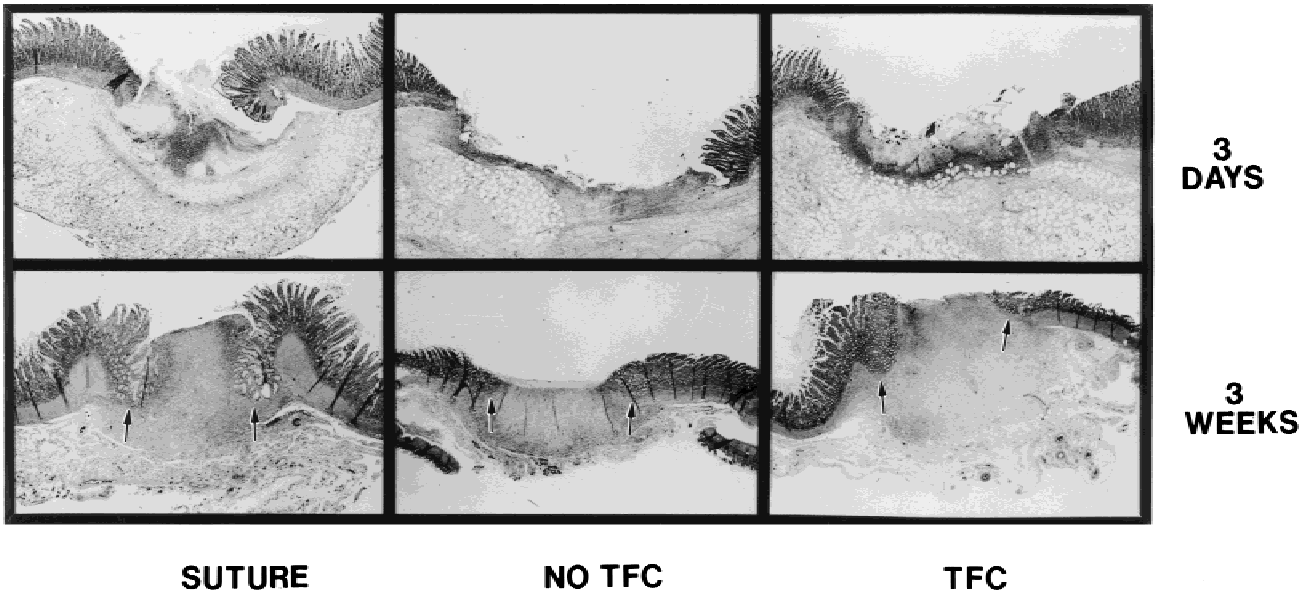

Fig. 4. Ho:YAG laser-assisted rat intestine anastomoses, 3 days and 3 weeks. At 3 days, the wound gaps are widened by slough of the necrotic wound edges in all specimens. At 3 weeks, the gaps are filled with fibrous (collagenous) scar tissue that originated from the bowel wall and the still adherent omentum. The irregular glands (arrows) mark the sites of mucosal regeneration. (Hematoxylin and eosin stain. Original magnification 6.25×)

the suture controls showed similar wound healing patterns, tissue composition, and progression with two exceptions [Table 4]. At 1 day, abscess formation and hemorrhagic necrosis were more prevalent in all Ho:YAG study groups, including the suture controls than the comparable argon groups. In addition, the wound gaps for the argon laser-assisted anastomoses were larger than those of the Ho:YAG group at 1 week. But by 3 weeks, the trend had reversed: the argon wound gaps became smaller having similar widths as the suture controls, whereas the Ho:YAG bond gaps remained larger.

DISCUSSION

All intact Ho:YAG laser-assisted intestinal welds were fluid-tight immediately after application of laser irradiation. However, by day 1, histologic examination showed that none of the thermally welded bond edges were fused but were separated forming gaps in the bond. Gaps also appeared in the suture control anastomoses, but since the bond was stabilized by several sutures, the gaps were generally smaller in the controls than the laser-assisted specimens. Most of the anastomoses, including the suture controls, were at least partially covered with adherent omentum, adjacent bowel, and/or mesentery that covered the variably sized wound gaps. The majority of the anastomotic complications occurred within the first 24–72 hours postoperatively. At autopsy, the failed anastomoses had very little adherent fatty tissue covering the bond sites. Since the bonds had been affected by infection (peritonitis) and postmortem autolysis, histologic evaluation of the bonds would have been without value and therefore were not done. However, no significant differences in the histologic features of wound healing were found in any of the intact laser-assisted and control anastomoses harvested during the first week. Therefore, no histopathologic factors could be identified that could explain the increased in vivo stability of the TFC laser-assisted anastomoses compared to those formed without TFC. We speculated that TFC stabilized laser welds by preventing excessive thermal buildup and by keeping the rate of tissue denaturation almost constant. Uncontrolled thermal buildup and thus excessive thermal damage may have reduced the speed of recovery during the initial healing phase of the laser welds performed without TFC.

The increasing mechanical strength of all anastomoses reflected by the increasing BLP is due to the formation of omental, bowel, and mesenteric adhesions around the anastomosis and laying down of fibrous wound tissue during the first week. As more fibrous (collagenous) scar tissue is formed in the wound gaps and adjacent

TABLE 4. Extent of Necrosis and Wound Gap Size: Comparisons of Argon and Ho:YAG Laser-Assisted Intestinal Anastomoses

	1 day[a] Necrosis [mm]	1 day Gap Width [mm]	3 days Gap Width [mm]	1 week Gap Width [mm]	3 weeks Gap Width [mm]
Argon laser group					
Suture control	0.87	0.17	1.51	0.75	1.89
Ho:YAG laser group					
Suture control	4.90[b]	3.08	0.57	3.18	1.72
Argon laser with temperature control	1.23	2.12	5.19	5.02	1.91
Ho:YAG laser with temperature control	1.58	0.87	5.02	3.49	3.32
Argon laser without temperature control	1.31	1.63	3.49	4.48	1.90
Ho:YAG laser without temperature control	1.73	1.36	4.53	2.24	3.30

[a]Necrotic tissue present at Day 1 is sloughed by Day 3.
[b]Measurements include coagulative and hemorrhagic necrosis.

adhesions, the anastomoses become stronger and more resistant to applied pressures. By 3 weeks, the laser-assisted anastomoses are just as strong as the sutured controls and intact normal small intestine.

Comparisons of wound healing and BLP measurements of argon ion laser-assisted intestinal welds from our previous study [2] with those of the Ho:YAG welds showed that: (1) the Ho:YAG bonds were stronger than argon welds at 3 days but weaker than the argon bonds at 1 week, and (2) argon welds healed faster than Ho:YAG laser-assisted welds. At 1 week, argon laser-assisted intestinal welds with TFC were almost as strong as the control sutured anastomoses, whereas the Ho:YAG welds were considerably weaker. At 3 weeks, the argon wound gaps equaled those of the suture controls, but the Ho:YAG gaps remained significantly larger reflecting a delay in healing. Histologic examination showed that the Ho:YAG experimental group including the suture controls had an increased incidence of abscess formation that distorted the adherent tissues and increased wound volumes. In some specimens, the walls of the abscesses were necrotic and thin, thus probably contributing to the mechanical weaknesses of the Ho:YAG laser-assisted bonds during the early healing period. In addition, the relatively larger Ho:YAG wounds associated with the abscesses required more time to fill in with scar tissue, thus delaying the final feature of intestinal wound healing, mucosal regeneration. Review of the operative techniques did not reveal any specific cause of the higher incidence of abscess formation in the Ho:YAG group compared to the argon group. Otherwise, no consistent differences in mechanical strengths or healing patterns were found that could be related specifically to wavelength or use of TFC.

The results of Poppas et al. [5] suggest that the use of an albumin solder stabilizes acute porcine cutaneous welds and permits welding at surface temperature as low as 65°C. We found it necessary to use surface temperatures above 80°C for laser welding of rat bowel without a solder. Our results are more similar to the constant temperature welding data of Stewart et al. [6] for rat aorta. Stewart et al. [6] did not use an albumin solder and reported acute success rates of 0 and 66% for control temperatures of 70°C and 75°C, respectively. Stewart et al. [6] did not have any significant difference in the acute bursting pressures for control temperatures of 75°C, 80°C, and 90°C. Although Stewart et al. [6] experiments involved 1 mm transverse cuts in the rat aorta, the overall results are in general agreement with our argon [2] and Ho:YAG results.

The results of Stewart et al., [6] our argon data [2], and this report note that the temperature control does not provide a satisfactory method for ending laser radiation. Rather, vague visual clues had been used in these studies to end the welding process. Thermal coagulation of tissues has been described by rate process theory as an accumulation of denatured proteins that is related linearly to time and exponentially to temperature [7]. If a certain degree of coagulation could be related to weld strength, then according to rate process theory, completion time for a weld at 90°C should be ~10 times less than weld time at 80°C. However, total procedure times for 1 mm transverse arteriotomies reported by Stewart et al. [6] were between 3–5 seconds for temperature from 75–90°C. Likewise, our in vitro canine intestine welding times using control temperatures from 80–95°C [1] did not show the extreme variations expected for a specified fraction of denaturation. We believe that constant temperature does

provide a more uniform rate of coagulation and can eliminate carbonization. Yet distinct differences between the rates of denaturation at 70–90°C have not been observed.

These results suggest several intriguing possibilities concerning the formation of successful thermal tissue welds. In the first place, other processes not defined by coagulation rate kinetics such as tissue desiccation may be factors in the formation of a successful weld. Second, the vague visual cues used as end points in these studies are not reliable indicators of the degree of tissue coagulation, but are an ill-defined endpoint for a successful weld. Third, to create more reproducible welds, it will be necessary to identify other measurable, probably nonthermal end points that will directly relate to a clinically successful weld. Clinical success is defined as formation of a stable, strong bond that does not deteriorate over time. More precise identification of end points that mark bond stability and strength should be experimental goals in the future.

CONCLUSIONS

This study evaluated a successful application of TFC as a dosimetry control modality in laser tissue welding. The findings indicated that TFC stabilized laser-assisted rat intestinal welds in vivo in the 24–72-hour postoperative period, as reflected by a decreased incidence of postoperative complications. However, in vitro bursting luminal pressure measurements showed great variation among the laser-assisted welds but no specific trends that could explain the in vivo stability of the TFC. Likewise, histopathologic examinations did not reveal any specific healing mechanisms that could be responsible for the increased stability. Comparisons of two different wavelengths (lasers) showed no differences, and variations could be attributed to nonspecific abscess formation and surgical technique. However, TFC does not provide a satisfactory method to identify the completion of a clinically successful weld. Using TFC to control rate of tissue coagulation, future studies should be done to identify end points that mark bond stability and strength.

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